

**REMARKS**

Upon reinstatement of the application, Applicants respectfully request entry of this Response to the Notice of Non-Compliant Amendment and Supplemental Response to the Office Action dated November 15, 2002 with Amendment. Applicants thank Examiner Portner for her help in resolving the outstanding issues regarding the prosecution of this case in a telephone conference that took place on January 6, 2004.

**Response to Notice of Non-Compliant Amendment**

The present communication is fully responsive to the Notice of Non-Compliant Amendment dated in the USPTO on April 22, 2003. The Notice of Non-Compliant Amendment alleges that the Amendment filed by the Applicants on April 16, 2003, is non-compliant because it does not adhere to the requirements for amendment provided in 37 C.F.R. § 1.121, specifically, the Amendment does not provide marked-up and clean versions of the replacement paragraphs as stipulated under 37 C.F.R. § 1.121(b)(1)(ii) and 37 CFR § 1.121(b)(1)(iii). In response to the Notice of Non-Compliant Amendment, Applicants have herein provided paragraphs appropriately amended under the *revised* 37 C.F.R. § 1.121 that became effective July 30, 2003. Accordingly, this Response provides marked-up replacement paragraphs indicating changes made relative to the originally filed versions; a clean copy of the paragraphs is not required and is not provided.

Applicants submit that entry of this Response remedies any alleged deficiencies in the Amendment dated April 16, 2003, so that the Amendment is now fully compliant with the requirements of 37 C.F.R. § 1.121. Accordingly, Applicants respectfully request entry of the Amendment dated April 16, 2003 in its entirety.

**Status of the Claims**

Following entry of the Amendment dated April 16, 2003 and the present Amendment, claims 1-4, 6, 12-14 and 34-56 are pending. Claim 15 is cancelled. Claims 1-4, 6, 12, 34-36, 38-42, and 45-46 are amended herein. Support for the claim amendments is found throughout the specification. These amendments introduce no new matter. These amendments are made without prejudice and are not to be construed as abandonment of the previously claimed subject matter or acquiescence to any objection or rejection of record.

Amendment to claims 4, 6, 36 and 38-40 are for the purpose of clarification or to correct typographical errors. Amendment to other claims are discussed below.

New claims 51-56 are provided in the present Amendment. The Examiner is respectfully requested to enter these new claims. These new claims do not constitute new matter and do not require burdensome additional searches by the Examiner. Support for these new claims is found throughout the Specification; the location of detailed support for the new claims is provided below.

**Claim 51** recites bacterial cells that express a *Moraxella bovis* cytotoxin or cytotoxin fragment from a recombinant nucleic acid. The use of bacterial cells for the expression of proteins from recombinant nucleic acid molecules is widely known in the art, and is taught in the specification, for example, in EXAMPLE 14 on page 47, EXAMPLE 18 on pages 50-52 and in EXAMPLE 24 at pages 61-62.

**Claim 52** recites cells that express a *Moraxella bovis* cytotoxin or cytotoxin fragment using an engineered expression vector. The use of expression vectors to express recombinant proteins is known in the art, and is taught, for example, in EXAMPLE 18 on pages 50-52 and in EXAMPLE 24 at pages 61-62.

**Claim 53** recites cells that express a *Moraxella bovis* cytotoxin or cytotoxin fragment from a recombinant nucleic acid and where the cytotoxin or cytotoxin fragment has a molecular weight of about 95 or 98 kDa. This claim is an edited version of previously cancelled claim 7. *M. bovis* cytotoxin species displaying these molecular weights are taught in the specification at page 18, line 27 through page 19, line 2; and see FIG. 6.

**Claim 54** recites cells that express a recombinant *Moraxella bovis* cytotoxin or cytotoxin fragment wherein the procedure to isolate the cytotoxin or cytotoxin fragment incorporates a gel filtration chromatography step wherein the diafiltered retentate of claim 4 is used as the starting material for the gel chromatography. The chromatography protocol is taught in EXAMPLE 10 on page 43; and see FIG. 2.

**Claim 55** recites fragments of the cytotoxin of SEQ ID NO: 2, where the fragments are a portion of the amino acid sequence of SEQ ID NO: 2 that are smaller than the full-length cytotoxin. The protocols required to express any desired fragment of a protein using recombinant DNA technology are well known and routine to one of skill in the art. Such protocols are amply described in many published molecular genetics laboratory guides.

The specification provides ample guidance in the construction of those cytotoxin fragments shorter than the full length cytotoxin that have desired properties, *e.g.*, fragments that find use as vaccines. A cytotoxin fragment can be defined by structural or functional criteria, as recited in new claim 55.

In one embodiment, **claim 55(a)**, a fragment is that portion that can induce antibody production in an animal, and the resulting antibody will specifically bind the full-length cytotoxin protein. Protocols for the production of antibodies (*e.g.*, in response to a cytotoxin fragment) is described, for example, in the specification in EXAMPLE 11 (pages 43-44) and EXAMPLE 19 (pages 52-53). Testing of the affinity of these anti-cytotoxin fragment antibodies (or the antisera) for the full length cytotoxin protein can be easily accomplished without undue experimentation using either the chromogenic immunoassay described in EXAMPLE 9 (pages 42-43) or the Western blot analysis described in EXAMPLE 15 (pages 47-48).

In other embodiments, a fragment of the cytotoxin is defined by the biological activity retained by the fragment. In one embodiment, **claim 55(b)**, a fragment is that portion that retains cytolytic activity, which is assayed using the lymphocyte cytolytic assay of either EXAMPLE 4 (pages 39-40) or EXAMPLE 5 (page 40). In another embodiment, **claim 55(c)**, a fragment is that portion that retains hemolytic activity, which can be assayed using a hemolysin (*i.e.*, erythrocyte lysis) assay in EXAMPLE 8 (page 42). In another embodiment, **claim 55(d)**, a fragment is that portion that retains corneolytic activity, which can be assayed by measuring corneal epithelial cell lytic activity, as described in EXAMPLE 13 (pages 45-47). In other embodiment, **claim 55(e)**, a combination of criteria can simultaneously be used to define a cytotoxin fragment.

The specification also teaches cytotoxin fragments comprising amino acids 438-713, **claim 55(f)**; amino acid 590-927, **claim 55(g)**; and 643-927, **claim 55(h)**; see the specification at page 17, lines 16-29; page 24, lines 13-20; page 33, lines 17-27; page 53, lines 3-6; and page 61, line 17 through page 62, line 13.

**Claim 56** recites fragments of the cytotoxin of SEQ ID NO: 2, where the fragments are a portion of the amino acid sequence of SEQ ID NO: 2 that are smaller than the full length cytotoxin and the fragments are immunogenic, *i.e.*, provide protective immunity to an animal following injection with the fragment. Assays for the prediction of immunogenicity

are provided in the specification. These assays are termed neutralization assays, and assess the *in vitro* ability of the anti-fragment antibodies to block the disease-causing activities of the cytotoxin fragment. These neutralization assays do not require undue experimentation and include lymphocyte lysis neutralization assays and the hemolysis neutralization assay (see, EXAMPLES 6 and 7, page 41; and EXAMPLE 21 on pages 53-55). A similar neutralization assay protocol can be used in the corneal cell lysis assay (see EXAMPLE 13 on pages 45-47).

Immunogenicity is also demonstrated in field trials using a cytotoxin fragment to immunize cattle against IBK, as described in the specification (see pages 33-34; and EXAMPLE 24 on pages 60-64). See especially page 34, lines 11-13, "Results of this field trial clearly show that the vaccine comprising recombinantly derived cytotoxin provided immunity against *M. bovis* in vaccinated animals."

Applicants assert that no new matter has been added to the application by way of the present Amendment. Entry of this Amendment is respectfully requested.

Support for amendments made to claims 1-4, 6, 12, 34-36, 38-42, and 45-46 is discussed in the section below.

#### Supplemental Response to Office Action with Amendment

Applicants herein provide a Supplemental Response to the Office Action dated November 15, 2002 with an Amendment. This Supplemental Response provides additional arguments to augment the arguments made in the Response filed on April 16, 2003. In this Supplemental Response, Applicants have also made amendment to the claims. Applicants respectfully request that the Examiner enter the Supplemental Response and reconsider the application in light of the supplemental arguments and amendments to the claims.

#### Rejections under 35 U.S.C. § 112, First Paragraph

In the Office Action dated November 15, 2002, claims 12-15 and 23-25 were rejected for alleged lack of enablement and written description. The Office Action alleges that the specification does not provide enablement or description for the formulation and use of *any* fragments of the *Moraxella bovis* cytotoxin (SEQ ID NO: 2).

Applicants respectfully disagree. Applicants point out that claims 15 and 23-25 have been cancelled, and claim 12 has been amended. The currently amended claim 12 is now dependent on claim 1, and is now directed to a cell that expresses a *Moraxella bovis* cytotoxin from a recombinant nucleic acid molecule.

In addition to the arguments provided in the Response filed April 16, 2003, Applicants point out that use of fragments of the full-length cytotoxin of SEQ IN NO: 2 is well supported in the specification (where a fragment is a cytotoxin polypeptide at least one amino acid shorter than the full length sequence of SEQ ID NO: 2). Fragments are readily constructed using molecular genetic engineering techniques well known to one of average skill in the art. As used herein, a cytotoxin fragment is defined by structural or functional criteria, as taught in the specification. For example, a fragment can be a portion that retains lymphocyte cytolytic activity, hemolytic activity or corneolytic activity. Assays for these activities are taught in the specification. The invention also provides examples of fragments with defined primary structures, for example, comprising amino acids 438-713, amino acid 590-927 or amino acids 643-927. See the discussion above regarding new claim 55 for more detailed descriptions of support.

Applicants assert that the recitation of cytotoxin fragments in the claims is enabled and sufficiently described in the specification to permit one to make and use cytotoxin fragments without undue experimentation. Applicants believe that claims reciting cytotoxin fragments are allowable, and accordingly, respectfully request withdrawal of this rejection.

Rejections under 35 U.S.C. § 102(a)

In the Office Action dated November 15, 2002, all pending claims were rejected for alleged anticipation by PCT publication WO 01/16172 to Farn *et al.*, published on March 8, 2001. The Examiner alleges that the cytotoxin provided by Farn *et al.* anticipates the cytotoxin and cytotoxin fragments provided by the present invention.

Applicants respectfully disagree. In addition to the arguments provided in the Response filed April 16, 2003, Applicants further point out that Farn *et al.* does not provide the cytotoxin of the present invention. The Applicants' invention provides a gene encoding a cytotoxin (see FIG. 3 and SEQ ID NO: 1) having a guanosine at nucleotide position 1096, whereas the PCT publication from Farn *et al.* provides a gene with an adenosine at that

position (see FIG. 5 and SEQ ID NO: 6 in that publication). This nucleotide difference results in the respective proteins have different amino acid sequences, where the Applicants' invention provides a cytotoxin with an alanine at amino acid position 366 (see SEQ ID NO: 2), whereas the cited PCT publication teaches a cytotoxin with a threonine at this position (see SEQ ID NO: 5 in that publication).

With regard to fragments, Farn *et al.* does not teach the production of the cytotoxin fragments provided by the Applicants invention, including SEQ ID NOS: 6 or 13 or any other fragment. In addition, Fran *et al.* does not teach a method for identifying cytotoxin fragments that induce the lysis of bovine lymphocyte cells or corneal epithelial cells, nor any means to determine whether a particular cytotoxin fragment is immunogenic, nor any method for generating isolated and purified cytotoxin or cytotoxin fragments.

Furthermore still, Applicants assert that the claimed invention was conceived and reduced to practice in the United States of America prior to the publication of Fran *et al.* on March 8, 2001. Applicants enclose an Affidavit of Inventor John A. Angelos under 37 C.F.R. § 1.131, with attached exhibits. These exhibits establish:

- 1) a scientific paper authored by inventors John A. Angelos, John F. Hess and Lisle W. George entitled "Cloning and characterization of a *Moraxella bovis* cytotoxin gene," was published in the American Journal of Veterinary Research, Vol. 62, No. 8, p. 1222-1228; August 2001;
- 2) the manuscript was submitted to AJVR for review on August 25, 2000, and cites GenBank Accession No. AF205359;
- 3) the GenBank Accession No. AF205359 provides the MbxA (*i.e.*, the cytotoxin) protein sequence identical to the sequence provided in Application Serial No. 09/884,696, SEQ ID NO: 2, and was first submitted to GenBank on November 15, 1999; and,
- 4) the GenBank Accession Number was held for release until August 2001 to coincide with publication of the AJVR article.

In view of the Affidavit establishing a date of invention that predates March 8, 2001, as outlined above, Applicants respectfully request the removal of PCT publication WO 01/16172 to Farn *et al.*, published on March 8, 2001, from consideration in the present case.

Rejections under 35 U.S.C. § 102(b)

In the Office Action dated November 15, 2002, all pending claims were rejected for alleged anticipation by PCT publication WO 90/07525, published on July 12, 1990; Billson *et al.*, *FEMS Microbiology Letters*, 124:69-74 (1994); and Billson *et al.*, *Infection and Immunity*, 68(6):3469-3474 (2000). The Action alleges that the cytotoxins taught in these references anticipate the cytotoxin and cytotoxin fragments provided by the present invention.

Solely for the purpose of advancing the prosecution of the present application, without acquiescing to the rejections in the Office Action, and reserving the right to prosecute the original or similar claims in the future, Applicants have amended claims 1, 12, 34, 41 and 45. These amended claims are no longer directed to a cytotoxin or cytotoxin fragment, *per se*, and are now directed to a cell that expresses a *Moraxella bovis* cytotoxin or cytotoxin fragment from a recombinant nucleic acid molecule, and where the expressed cytotoxin or cytotoxin fragment is specifically bound by an antibody that specifically binds to a full-length cytotoxin.

Applicants point out that the cited references WO 90/07525; Billson *et al.*, (1994); and Billson *et al.*, (2000) clearly can not teach this presently claimed subject matter because the cited references do not teach a recombinant nucleic acid molecule that expresses the cytotoxin of SEQ ID NO: 2 or a fragment thererof.

Support for the amendment to claim 1, and the claims dependent on claim 1, is found throughout the specification. Testing whether a cytotoxin or cytotoxin fragment is specifically bound by an anti-cytotoxin antibody (or antisera) can be easily assessed without undue experimentation using either the chromogenic immunoassay described in EXAMPLE 9 (pages 42-43) or the Western blot analysis described in EXAMPLE 15 (pages 47-48).

Cytotoxin fragments are also supported in the specification. Cytotoxin fragments are readily constructed, and can be defined by structural or functional criteria, as taught in the specification. For example, a fragment can be a portion that retains lymphocyte cyolytic activity, hemolytic activity or corneolytic activity. Assays for these activities are taught in the specification. The invention also provides examples of fragments with defined primary structures, for example, comprising amino acids 438-713, amino acid 590-927 or amino acids

643-927. See the discussion above regarding new claim 55 for detailed descriptions of support.

Claim 1 also encompasses cells expressing modified variants of the cytotoxin provided in GENBANK Accession Number AF205359. The specification teaches a number of variants of the cytotoxin polypeptide, as evidenced by multiple forms of the cytotoxin observed in Western blots, including for example, as shown in FIG. 2 (see page 13, line 11 through page 14, line 5) and the Western blots of FIGs. 5 and 6 (see page 18, line 9 through page 19, line 14). These FIGs show multiple variants of the MbxA cytotoxin, as evidenced by their specific reactivity with an anti-cytotoxin antibody.

In the Office Action dated November 15, 2002, claims 12-15 and 23-25 were rejected for alleged anticipation by CA2014033-A, published October 7, 1990; US Patent No. 5,475,098, issued December 12, 1995; and CA2170839, published September 2, 1996. The Office Action alleges that the cytotoxins taught in these references anticipate the cytotoxin and cytotoxin fragments provided by the present invention.

Solely for the purpose of advancing the prosecution of the present application, without acquiescing to the Examiner's arguments, and reserving the right to prosecute the original or similar claims in the future, Applicants have cancelled claims 15 and 23-25, and amended claim 12. Applicants point out that amended claim 12 is now dependent on claim 1, and no longer directed to a cytotoxin or cytotoxin fragment, *per se*, and is now directed to a cell that expresses *Moraxella bovis* cytotoxin from a recombinant nucleic acid molecule.

Applicants point out that the cited references CA2014033-A; US Patent No. 5,475,098; and CA2170839 do not teach the presently claimed invention as currently amended. The cited references do not teach a fragment of a recombinant *Moraxella bovis* cytotoxin where the fragment is expressed by a recombinant nucleic acid molecule encoding the cytotoxin of SEQ ID NO: 2 or a fragment thereof, where the fragment is specifically bound by an antibody that specifically binds a full-length *Moraxella bovis* cytotoxin encoded by SEQ ID NO: 2 (the *Moraxella bovis* cytotoxin). The Office Action provides amino acid sequence alignments that contain at most a six amino acid fragment of contiguous amino acid identity between the sequences cited in the rejection and the MbxA cytotoxin amino acid sequence. It is unlikely that a single six amino acid portion of a protein will impart antibody cross-reactivity with a second protein.

Applicants assert that the subject matter recited in amended claims 1 and 12 and previously presented claims 13 and 14 is not anticipated, and are allowable. Accordingly, Applicants respectfully request withdrawal of the rejection.

### CONCLUSION

In view of the foregoing, and following entry of the Amendment dated April 16, 2003 and the present Amendment, Applicants believe all pending claims, including the newly added claims, in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the claims are deemed not to be in condition for allowance after consideration of this Response, a telephone interview with the Examiner is hereby requested to expedite the prosecution of this case. Please telephone the undersigned at (510) 337-7871 to schedule an interview.

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Respectfully submitted,



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Attachments:

- 1) Petition to Withdraw Holding of Abandonment Based on Failure to Receive Office Action;
- 2) Affidavit of Hana Verny;
- 3) Contingent Petition for Revival of an Application for Patent Abandoned Unintentionally;
- 4) Affidavit of Inventor John A. Angelos with Exhibits A-D;
- 5) A transmittal sheet;
- 6) A fee transmittal sheet; and,
- 7) A receipt indication postcard.